BIOLOGICAL SIGNATURES IN CARBONATES: YELLOWSTONE NATIONAL PARK. Carlton C. Allen[1], Catherine R. Graham[2], Joan Combie[3], Fred G. Albert[3], Andrew Steele[4] and David S. McKay[4] [1]Lockheed Martin, Houston, TX 77258 carlton.c.allen1@jsc.nasa.gov [2]Brown University, Providence, RI 02912 [3]Montana Biotech, Belgrade, MT 59714 [4]NASA Johnson Space Center, Houston, TX 77058.

Evidence of possible relict biogenic activity has been reported in carbonate inclusions within martian meteorite ALH84001 [1]. We are studying evidence of contemporary biogenic activity in a possible terrestrial analog, the high temperature carbonate thermal springs of Italy and the United States [2,3]. We are examining the thermophilic microorganisms which live in those environments and the preservation of viable and fossilized microbes within thermal spring carbonate deposits.

Mammoth Hot Springs is a 4 x 1.4 km terraced mound of calcium carbonate located in Yellowstone National Park. Carbonate deposition (aragonite and calcite) is ongoing from streams fed by an array of hydrothermal vents. The streams support a diverse biota dominated by microbes which are segregated according to local conditions including temperature, pH and water composition [4]. Carbonate deposition in many places is rapid enough to trap and preserve evidence of microbial life.

We collected samples of carbonate, water and microbes from the hottest portions of Narrow Gauge springs, an active group of sulfide and carbonate springs in the northwest portion of the Mammoth complex. Water temperatures measured at the vents ranged from 66°C to 72°C and pH from 6.3 to 6.5. Chafetz and Lawrence [5] reported similar conditions, and noted that water emerges from the vents supersaturated with respect to aragonite.

The dominant species immediately downstream from the vents has been identified as *Thermothrix thiopara*, a fast-growing sulfur-oxidizing bacterium [6]. *Thermothrix* produces cream-colored filaments up to 10 cm long oriented parallel to the water flow. While initially identified as a single species [7], unpublished genetic studies of *Thermothrix* filaments show evidence of two distinct types of bacteria within the filaments [D. Brannan, pers. comm.].

A few tens of centimeters downstream from the vents, as the water begins to cool and degas, *Thermothrix* filaments become overgrown by aragonite [8]. The result is a mass of aragonite needles, each millimeters in diameter and centimeters long. The needles are oriented parallel to the water flow and closely mimic *Thermothrix* filaments. Thus, bacterially-induced precipitation yields a distinctive petrographic fabric in carbonate rocks. This "streamer" fabric has been recognized in deposits as old at early Carboniferous (~360 my) [8].

We are concentrating on aragonite needles as the highest-temperature mineralization in carbonate thermal springs. These structures contain viable microbes, biofilm and possible nanobacteria.

Viable Bacteria. We dissected aragonite needles, etched them lightly in 0.1% HCl and examined them in a high resolution SEM. No intact cells were found following several extensive searches.

Aragonite needles were flamed to destroy surface contamination, then crushed and incubated in a nutrient medium chosen to culture thermophilic bacteria [9]. After 20 hrs at 60°C the cultures produced limited numbers of 3-4 µm long rod-shaped microbes (Fig. 1) and other 1 µm long rods. We have initiated an extensive set of experiments and analyses to identify these microbes.

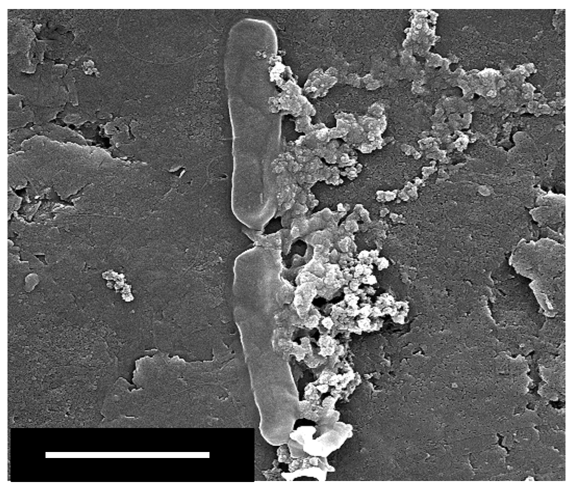

Figure 1. Microbes cultured from aragonite needles; 2 µm scale bar

Biofilm. The aragonite commonly contains masses of organic mucus, concentrated near the needles' long axes. This mucus is characteristic of many bacterial mats, where it binds the cells into a biofilm. The film coats the surfaces of individual aragonite crystals, indicating that mucus was being formed even after carbonate crystallization (Fig. 2).

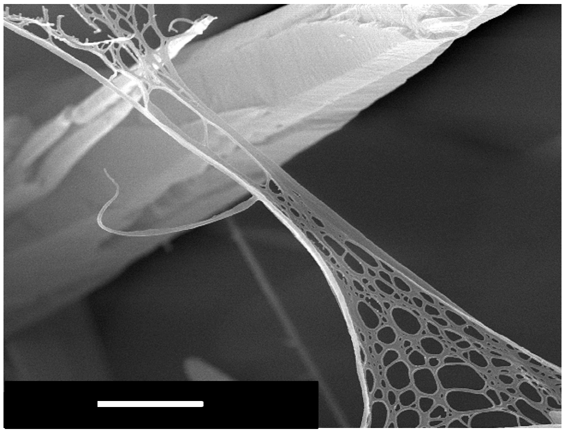

Figure 2. Organic mucus on aragonite crystal; 1 μm scale bar

Nanobacteria (?) Folk [10] reported <200 nm diameter, acid-resistant spheres in thermal spring carbonate deposits from Italy. He argued that these spheres are viable organisms, which he called nanobacteria since they are significantly smaller than the commonly-accepted lower dimension for bacteria.

The aragonite filaments from Narrow Gauge contain abundant 100 to 200 nm spheres which closely resemble those described by Folk. The spheres are composed of C, O, F, P and Ca with detectable Si and S, and resist dissolution in 0.1% HCl. They are invariably found enmeshed in organic mucus (Fig. 3).

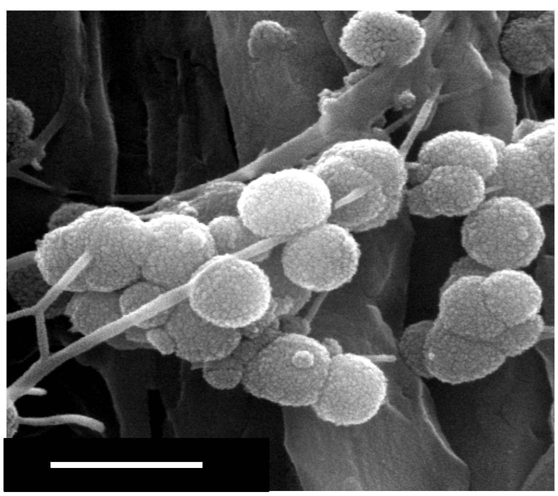

Figure 3. Nanobacteria (?) in organic mucus; 500 nm scale bar

We have not yet determined whether these spheres are organisms, spores, fossils or inorganic precipitates. *Thermothrix thiopara* is not a spore-forming bacterium but culturable spore formers have been found at the Jemez hot spring in New Mexico (T. L. Kieft, pers. comm.). We are currently attempting to isolate the spheres, quantify their composition, culture the isolates and test for DNA.

Preservation. Cells are poorly preserved in our aragonite samples. Farmer and Des Marais [8] noted that organic matter is rare in Yellowstone carbonates deposited at temperatures <30°C, suggesting that decomposition rates in such thermal environments are very high.

In rare cases the central core of an aragonite needle preserves the remains of a *Thermothrix* filament. The biofilms also contain the remains of small rod-shaped microbes (Fig. 4) which have been partially dissolved.

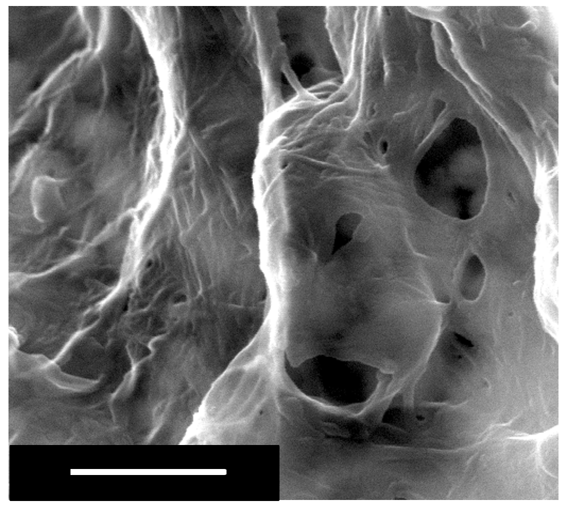

Figure 4. Microbial remains incorporated in biofilm; 500 nm scale bar

Conclusions. Carbonate thermal springs support several species of thermophilic microbes at temperatures as high as 72°C. These may be rapidly fossilized but are poorly preserved. Organic biofilms and possible nanobacteria, as well as morphologically and mineralogically distinctive carbonate rock fabrics, are more enduring biomarkers.

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